High-Affinity Disaccharide Binding by Tricyclic Synthetic Lectins

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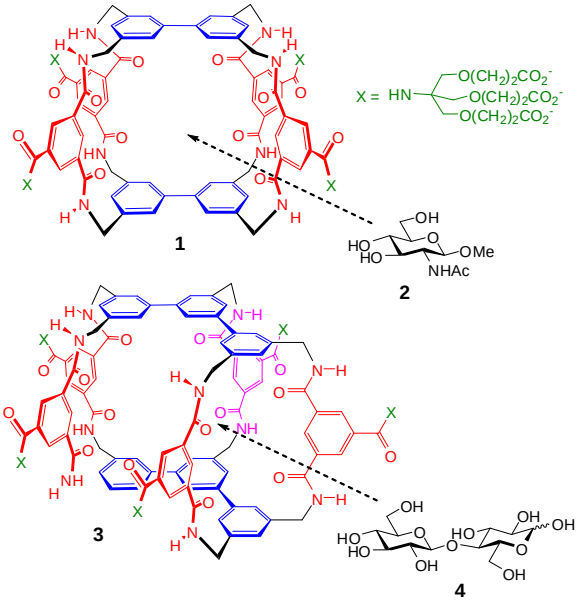
((Dedication----optional))

Carbohydrate recognition mediates a wide range of biological processes,[1] including protein folding and trafficking,[2] cell-cell recognition,[3] infection by pathogens,[4] and many aspects of the immune response.[5] Molecules capable of selective carbohydrate binding are therefore valuable as tools for biological research, and potentially as medicinal agents. [6,7] There are many saccharide-binding proteins, notably the group known as lectins,[8] but they often show low affinities and (from a researcher's viewpoint) non-ideal selectivities.[8,9] Moreover they are generally too unstable and toxic for use in medicine. Opportunities thus exist for synthetic systems,[10] provided they can compete with lectins in binding strength and selectivity.

Over the past few years we have shown that macropolycyclic lactams such as **1** and **3** (Scheme 1) can bind carbohydrates with all-equatorial substitution patterns in water, employing a combination of hydrogen bonding and hydrophobic/CH-π interactions.[11] Selectivities compare well with those of lectins; for example, both **1** and **3** bind their respective targets **2** and **4** with preferences of >20:1 vs. closely-related substrates.[11b,c] However affinities have been less competitive. While lectins typically bind monosaccharides and disaccharides with K_a = 10^3-10^5 M^{-1} [8,9] the values for **1** and **3**, at ~600 M^{-1}, lie short of this range. We now report two new synthetic lectins **5** and **6**, related to tetracycle **3** but with a less preorganised and more accessible architecture. Remarkably these systems yield *increased* performance in key respects, with K_a up to 4500 M^{-1} and extreme selectivity for di- *vs.* mono-saccharides. This work provides the best evidence yet that true lectin mimicry may be achieved with simple, practically viable receptor structures.

The decision to undertake this study was made via an indirect route which nicely illustrates the limitations of current molecular design. When initially we planned to target all-equatorial disaccharides, tricyclic system **5** seemed an obvious solution.[12] However, modelling[13] showed that **5** can undergo a twisting motion which brings together the central aromatic rings in each terphenyl unit (Figure 1). In water, this collapsed conformation was predicted to be ≥25 kJ mol^{-1} more stable than open structures, presumably compromising disaccharide binding. We therefore



Scheme 1. Previously reported macropolylactam synthetic lectins **1** and **3**, with favoured substrates D-GlcNAc-β-OMe **2** and D-cellobiose **4**. Binding is driven by hydrogen bonding to equatorial polar groups in the carbohydrates, and hydrophobic/CH-π interactions to axial substrate CH.

turned to the more highly-connected **3** which, as previously described,[11b] proved very effective.

Despite the success of **3**, we remained curious whether the rigid tetracyclic architecture was necessary or, alternatively, the tricyclic (and synthetically more accessible) **5** might show at least moderate binding properties. Having recently found that biphenyl 4,4'-alkoxy substituents could improve affinities in monosaccharide receptors,[11d] we were also interested to test the dimethoxy analogue **6**. We therefore synthesized **5** and **6** from haloarenes **7** via sequential Suzuki-Miyaura cross-couplings, deprotections and macrolactamisations as shown in Scheme 2.[14] The methodology was largely based on earlier work, but a new development was the use of diazido aryl halides **8** as coupling partners for boronates **9**. While care was required to prevent azide degradation during coupling, the Staudinger reduction of tetra-azido macrocycles **11** proceeded especially smoothly. This protection strategy proved superior to alternatives such as Cbz, for which removal could be problematic.

Macrocycles **5** and **6** were examined first by ^{1}H NMR spectroscopy in D_2O. The parent system **5** gave broadened signals which could not be used for binding studies, but fortunately the tetra-methoxy analogue **6** gave well-resolved spectra. Dilution of an NMR sample from 0.5 mM to 0.15 mM caused negligible changes to the spectra, implying that **6** does not aggregate in this concentration range. Carbohydrate recognition by **6** was then studied by ^{1}H NMR

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[**] This work was supported by the Royal Thai Government (Fellowship to B.S.), the Engineering and Physical Sciences Research Council (grant number EP/D060192/1) and the Royal Society (Newton International Fellowship to C.K.)

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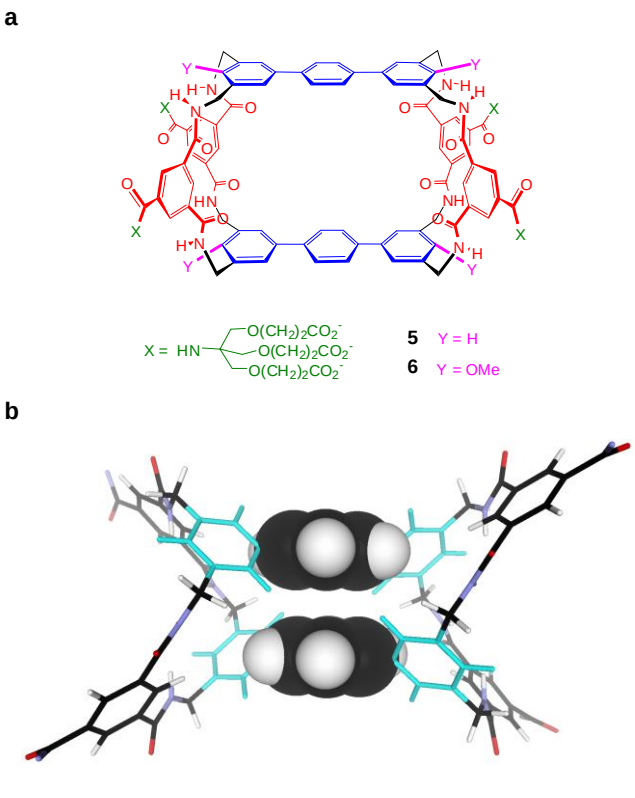


Figure 1. a) Formulae for receptors **5** and **6**, discussed in this paper. b) Ground state conformation of **5** as predicted by Monte Carlo Molecular Mechanics (MCMM).[13,14] The two ends of the molecule have twisted relative to each other, bringing the central terphenyl aromatic rings into close proximity. The central rings are shown in space-filling mode, and the terminal terphenyl aromatics are highlighted in cyan. Water-solubilising groups are omitted for clarity.

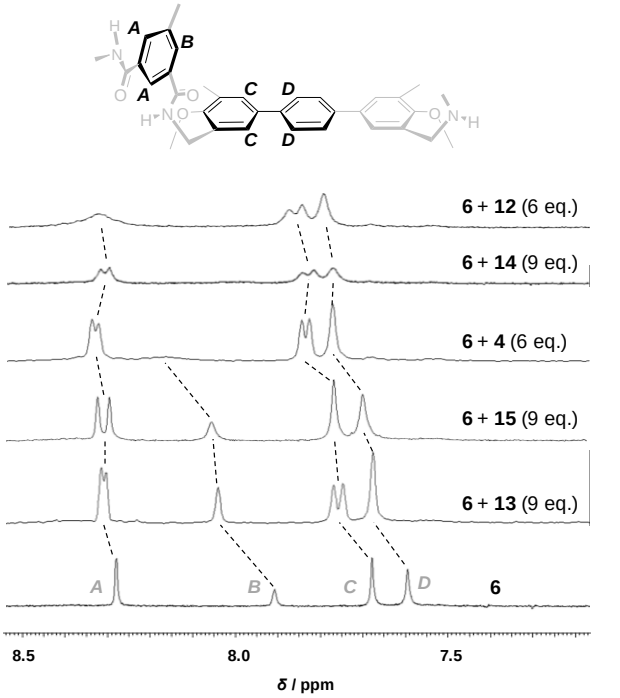
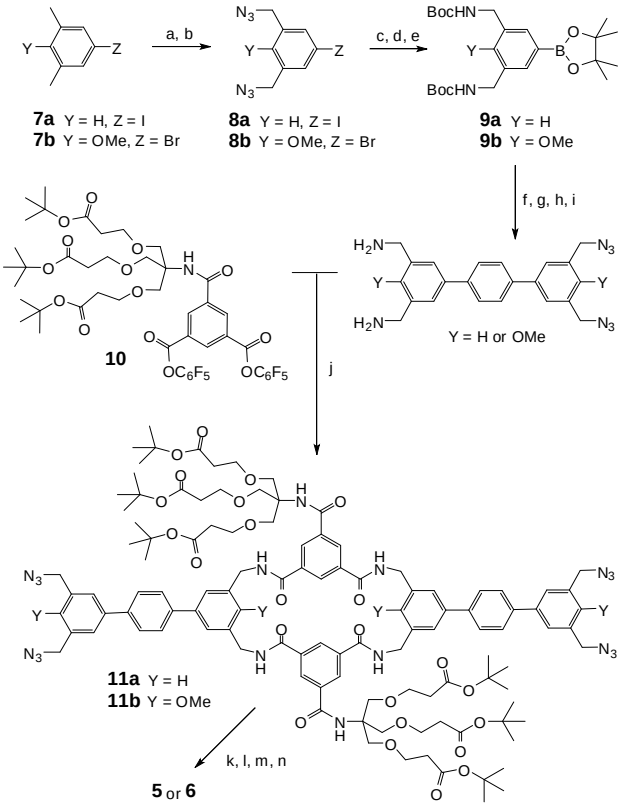


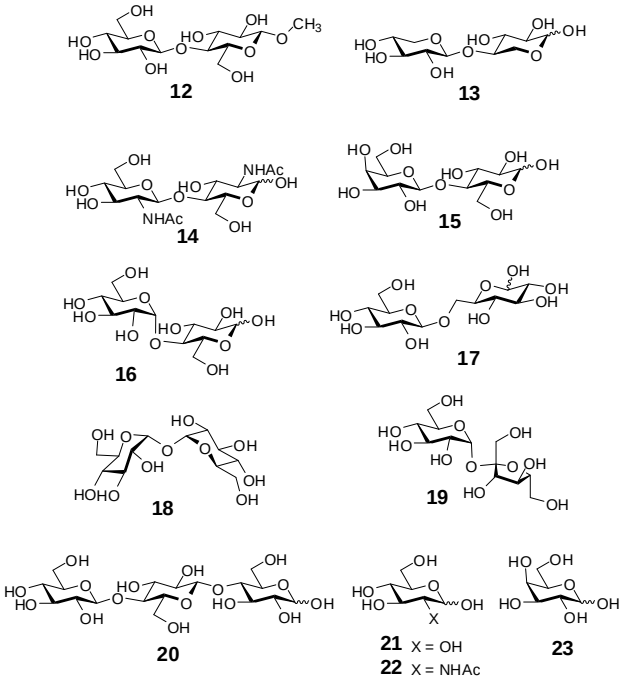
Figure 2. Assigned partial ^{1}H NMR spectra (500 MHz, D_2O, 298 K) of **6** alone (0.5 mM) and with added methyl β-D-cellobioside **12**, *N,N'*-diacetyl-D-chitobiose **14**, D-cellobiose **4**, D-lactose **15**, and D-xylobiose **13**. Correlations between signals are indicated by dotted lines.



Scheme 2. Synthesis of receptors **5** and **6**. Yields refer to Y=H/ Y=OMe respectively: a) *N*-bromosuccinimide, benzoyl peroxide (cat.), MeOAc, 85°C, 48 h, 40/99%; b) NaN_3, DMF, 60°C, 24 h, ~99%; c) PPh_3, THF/H_2O, 60°C, 24 h, 95/99%; d) Boc_2O, DIPEA, THF, r.t., 24 h, 75/83%; e) pin_2B_2, $PdCl_2$(dppf) (3 mol %), KOAc, DMSO, 80°C, 18 h, 80/83%; f) 4-bromo-iodobenzene, $PdCl_2$(dppf) (3 mol %), Na_2CO_3, DMSO, 50°C, 24 h, 85%; g) pin_2B_2, $PdCl_2$(dppf) (3 mol %), KOAc, DMSO, 80°C, 2 d, 85/94%; h) bisazide **8a** or **8b**, $PdCl_2$(dppf) (3 mol %), Na_2CO_3, DMF, 80°C, 4 h, 72/74%; i) TFA, DCM, 0°C, 5 h, 78/89%; j) DIPEA, THF, 30 h injection, r.t., 24 h, 20/52%; k) PPh_3, THF/H_2O, 60°C, 24 h, 78/91%; l) **10**, THF, 30 h injection, r.t., 24 h, 29/58%; m) TFA, DCM, 0°C, 5 h, 98/99%; n) NaOH aq., 95/98%. DIPEA = *N,N*-diisopropylethylamine, pin_2B_2 = bispinacolato diboron, $PdCl_2$(dppf) = dichloro diphenylphosphinoferrocene palladium(II) complex.

titrations, using cellobiose **4** and saccharides **12**–**23** (Scheme 3) as substrates. Addition of **4** and several other substrates caused movements of signals in the aromatic region of the spectrum, implying binding with fast-medium exchange on the ^{1}H NMR chemical shift timescale (see Figure 2). Broadening was observed for some signals in some cases, but at least one proton could always be followed easily throughout a titration. The movements gave excellent fits to a 1:1 binding model, and were analysed to give binding constants K_a as listed in Table 1. Interestingly, the signals for aromatic protons *A* and *C* were split in some cases, while those for *B* and *D* remained singlets. This is consistent with the D_{2h} symmetry and conformational properties of the host. A carbohydrate entering the host renders all protons different but, in fast exchange, movement between four substrate orientations results in equivalence for groups of 4 protons. Thus *B* (4 protons) remains a singlet, while *A* (8 protons) and *C* (8 protons) are split into two groups of 4. The 8 protons of *D* appear as a singlet, because the two environments generated by the carbohydrate are exchanged by rotation of the C_6H_4 unit.

To support the NMR studies on **6**, we also employed fluorescence titrations and Isothermal Titration Calorimetry (ITC).



Scheme 3. Carbohydrates used as substrates for binding studies.

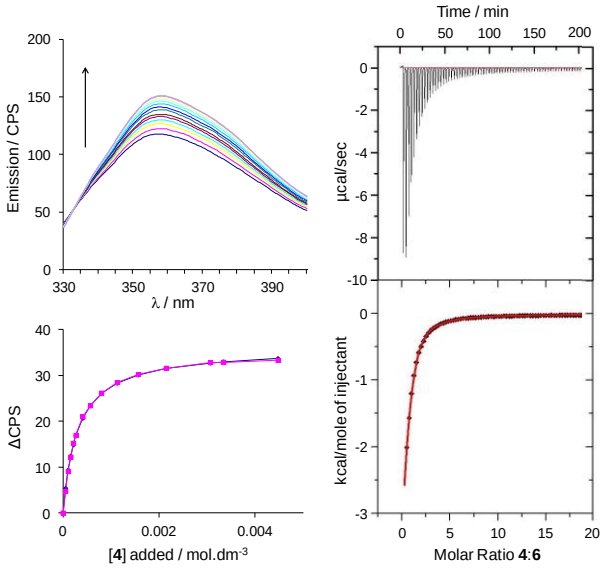


Figure 3. Data and analysis curves for (left) fluorescence and (right) ITC binding studies on receptor **6** + cellobiose **4** in H_2O at 298 K.[14] Both analyses employ a simple 1:1 binding model. K_a = 3330 and 3300 M^{-1} respectively.

Addition of carbohydrates to **6** in water caused increases in fluorescence output which, though modest, fit well to a 1:1 binding model (for example, see Figure 3). The K_a values obtained by this method are compared to the NMR-derived figures in Table 1. ITC traces (e.g. Figure 3) were also consistent with 1:1 complexation,[14] giving the thermodynamic parameters shown in Table 2. The fluorescence and ITC techniques were also applicable to parent tricycle **5**. Receptor **5** behaved similarly to **6** in these experiments, giving binding constants as listed in Tables 1 and 2 respectively. For both **5** and **6**, the agreement between different methods was excellent, especially for stronger-binding substrates. For example, in the case of **6** + **4**, the NMR, fluorescence and ITC measurements gave K_a = 3340, 3330 and 3300 M^{-1} respectively.

Table 1. Association constants (K_a, M^{-1}) for 1:1 complexes of receptors **5** and **6** with carbohydrate substrates in water, as determined by ^{1}H NMR and fluorescence titrations. Data for **1** and **3** are given for comparison.[a]

Carbohydrate	K_a [M^{-1}]				
	1	**3**	**5**[b]	**6**[c]	**6**[b]
methyl *β*-D-cellobioside **12**		850		4500	4470
D-cellobiose **4**	17	580	3140	3340	3330
D-xylobiose **13**		270		210	
N,N'-diacetyl-D-chitobiose **14**	~0	120		910	
D-lactose **15**	~0	14	230	270	240
D-maltose **16**	~0	11	67	72	80
D-gentiobiose **17**		5		35	36
D-trehalose **18**		~0		~0	~0
D-sucrose **19**		~0		~0	~0
D-cellotriose **20**				87	
D-glucose **21**	9	11[d]		2	3
N-acetyl-D-glucosamine **22**	56	24		9	9
D-galactose **23**	2			~0	

[a] T = 298 K unless otherwise stated. Values for **1** and **3** from refs [11a,b] respectively. Values denoted ~0 were too small for analysis. Values for reducing sugars are weighted averages of those for the two anomers, as discussed in ref [11d]. [b] Fluorescence titration in H_2O. Errors estimated at ≤ 4%. [c] ^{1}H NMR titration in D_2O. Errors estimated at ≤ 5%. [d] T = 278 K.

Considering the binding constants in Tables 1 and 2, two features stand out. Firstly, against our expectations, the affinities of both **5** and **6** for their target substrates **4** and **12** are considerably higher than achieved by the more rigid system **3**. Removing the 5th isophthalamide bridge, straightening the terphenyl units and allowing the framework to flex has strengthened binding by factors of 5 – 6. Indeed the affinity of **6** for methyl cellobioside **12**, at 4500 M^{-1}, is the highest yet observed for a synthetic receptor binding an uncharged carbohydrate substrate in water. Perhaps surprisingly, given our previous experience with monosaccharide receptors,[11d] the methoxy substituents made little difference; binding constants to **5** and **6** were almost identical. Secondly, the selectivity between disaccharides has been reduced (e.g. cellobiose:lactose = 40 for **3**, 13 for **6**), but the preference for disaccharides *vs.* monosaccharides has been increased. Thus the cellobiose:glucose selectivity has risen from 50 for **3** to ~1300 for **6**. Selectivity between di- and monosaccharides may not be especially difficult to achieve, but again this system sets new records.

An advantage of the generally high affinities is that accurate ITC measurements are feasible for **5** and **6** with both target and non-target substrates. As shown in Table 2, binding is driven by both enthalpy and entropy changes, but with enthalpy-entropy compensation such that enthalpy dominates for strongly-bound complexes. Comparison with literature data[9] shows that these figures are within the bounds observed for lectins, although with entropy contributions which are above-average for the natural systems. An enthalpy-entropy plot based on Table 2 is almost linear (see Figure S69, Supporting Information), as observed for other closely-related host-guest pairings.[15]

Structural aspects of binding were investigated by NMR and molecular modelling. We focused especially on **6** + **12**, which form a strongly bound complex and avoid the complications caused by substrate anomers. Intermolecular NOESY cross peaks provided clear evidence that, as expected, **12** enters the cavity of **6** to make hydrophobic/CH-π contacts with the

Table 2. Association constants (K_a, M^{-1}) and thermodynamic quantities (kJ mol^{-1}) for 1:1 host/guest complexes between receptors **3**, **5** and **6** and carbohydrate substrates in water at 298 K, as measured by ITC.

complex	K_a	ΔH	$T\Delta S$	ΔG
3 + cellobiose **4** [a]	650	-13.46	2.59	-16.05
5 + cellobiose **4**	3,110	-18.37	1.52	-19.89
5 + lactose **15**	220	-8.41	4.90	-13.30
5 + maltose **14**	61	-3.60	6.82	-10.42
6 + cellobiose **4**	3,300	-18.86	1.23	-20.08
6 + lactose **15**	250	-9.82	3.82	-13.64
6 + maltose **14**	89	-4.45	6.39	-10.83

[a] From ref [11b].

terphenyl units. Thus strong interactions were observed between axial substrate C*H* and receptor protons *C* and *D* (see Figure 2 for labelling), while cross-peaks from *C*/*D* to the C(6) protons were weaker. In particular, a correlation between cellobioside MeOC*H* and one receptor proton *C* places the methyl glycosidic unit unambiguously in one corner of the cavity. MCMM calculations generated a number of conformations consistent with these data, as exemplified in Figure 4. Typically these feature 4–6 intermolecular hydrogen bonds and ~10 CH-π interactions. Molecular dynamics simulations predicted that the complex should stay intact for at least 10 ns at 300 K.[14]

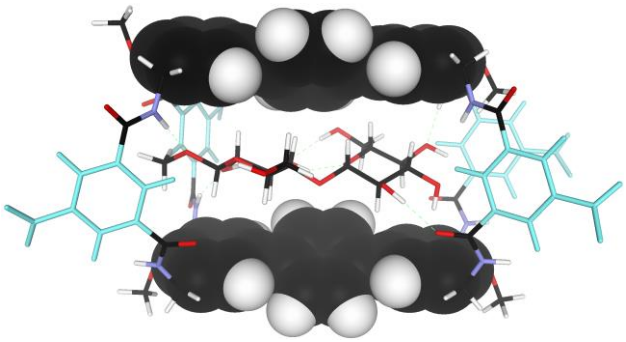


Figure 4. Possible structure for complex **6•12** derived from molecular modelling. Shown is the ground state geometry from an MCMM search in which both receptor and substrate were allowed conformational freedom.[14] Terphenyl aromatics are shown in space-filling mode, isophthalamide aromatics are coloured cyan. Water-solubilising groups are omitted for clarity.

It is interesting to compare the structure in Figure 4 with the NOESY-based model previously obtained for **3•4**. Unsurprisingly, given the additional spacer unit in **3**, the latter complex features more intermolecular hydrogen bonds (~10). On the other hand, the linear *p*-terphenyl unit in **6** may be slightly more compatible with the cellobiose CH than the bent, *m*-substituted system in **3** (see Figures S74/75, Supporting Information). Thus the strength of binding to **5** and **6** tends to reinforce our view that apolar hydrophobic/CH-π interactions provide the major driving force for carbohydrate recognition in water.[16] Whatever the reason, it is remarkable that **5** and **6** can outperform **3** despite the (at most) transient nature of their cavities (*cf.* Figure 1).

In conclusion we have shown that tricyclic synthetic lectins **5** and **6** are even more effective than tetracyclic **3** at binding all-equatorial disaccharides under biomimetic conditions. The success of these less-connected structures suggests that an "induced fit" or "conformational selection"[17] approach can be superior to rigid preorganisation in carbohydrate recognition, and may point the way to new, even simpler systems with potential for applications. In particular the disaccharide substrates are representative of major biopolymers (cellulose, xylan, chitin), and receptors which bind the polymers themselves could have biological activity or serve as aids to processing (e.g. by promoting solubility). Future studies will focus on these possibilities.

[1] *The Sugar Code - Fundamentals of Glycoscience* (Ed.: H.-J. Gabius), Wiley-Blackwell, Weinheim, **2009**. H. J. Gabius, H. C. Siebert, S. Andre, J. Jiménez-Barbero, H. Rudiger, *ChemBioChem* **2004**, *5*, 740. R. A. Dwek, T. D. Butters, *Chem. Rev.* **2002**, *102*, 283. C. R. Bertozzi, L. L. Kiessling, *Science* **2001**, *291*, 2357.

[2] J. Roth, *Chem. Rev.* **2002**, *102*, 285.

[3] S.-i. Hakomori, K. Handa, in *Carbohydrates in Chemistry and Biology* (Eds.: B. Ernst, G. W. Hart, P. Sinay), Wiley-VCH, Weinheim, **2000**, p. II 771.

[4] T. Angata, A. Varki, *Chem. Rev.* **2002**, *102*, 439.

[5] R. Kannagi, in *Carbohydrate-Based Drug Discovery* (Ed.: C.-H. Wong), Wiley-VCH, Weinheim, **2003**, p. 803.

[6] J. Balzarini, *Nat. Rev. Microbiol.* **2007**, *5*, 583.

[7] S. Jin, Y. F. Cheng, S. Reid, M. Y. Li, B. H. Wang, *Med. Res. Rev.* **2010**, *30*, 171.

[8] M. Ambrosi, N. R. Cameron, B. G. Davis, *Org. Biomol. Chem.* **2005**, *3*, 1593. H. Lis, N. Sharon, *Chem. Rev.* **1998**, *98*, 637.

[9] E. J. Toone, *Curr. Opin. Struct. Biol.* **1994**, *4*, 719.

[10] For reviews see ref [7] and: D. B. Walker, G. Joshi, A. P. Davis, *Cell. Mol. Life Sci.* **2009**, *66*, 3177. M. Mazik, *Chem. Soc. Rev.* **2009**, *38*, 935. T. D. James, M. D. Phillips, S. Shinkai, *Boronic Acids in Saccharide Recognition*, RSC, Cambridge, **2006**. A. P. Davis, R. S. Wareham, *Angew. Chem. Int. Ed.* **1999**, *38*, 2978. For recent examples see: G. Fukuhara, Y. Inoue, *J. Am. Chem. Soc.* **2011**, *133*, 768. A. Pal, M. Berube, D. G. Hall, *Angew. Chem. Int. Ed.* **2010**, *49*, 1492. M. Rauschenberg, S. Bomke, U. Karst, B. J. Ravoo, *Angew. Chem. Int. Ed.* **2010**, *49*, 7340. S. Striegler, M. G. Gichinga, *Chem. Commun.* **2008**, 5930. T. Reenberg, N. Nyberg, J. O. Duus, J. L. J. van Dongen, M. Meldal, *Eur. J. Org. Chem.* **2007**, 5003. H. Goto, Y. Furusho, E. Yashima, *J. Am. Chem. Soc.* **2007**, *129*, 9168. M. Mazik, H. Cavga, *J. Org. Chem.* **2006**, *71*, 2957.

[11] a) E. Klein, M. P. Crump, A. P. Davis, *Angew. Chem. Int. Ed.* **2005**, *44*, 298. b) Y. Ferrand, M. P. Crump, A. P. Davis, *Science* **2007**, *318*, 619. c) Y. Ferrand, E. Klein, N. P. Barwell, M. P. Crump, J. Jiménez-Barbéro, C. Vicent, G. J. Boons, S. Ingale, A. P. Davis, *Angew. Chem. Int. Ed.* **2009**, *48*, 1775. d) N. P. Barwell, M. P. Crump, A. P. Davis, *Angew. Chem. Int. Ed.* **2009**, *48*, 7673.

[12] We had previously found that a lipophilic analogue of **5** binds an organic-soluble cellobiose derivative in chloroform-methanol mixtures. See: G. Lecollinet, A. P. Dominey, T. Velasco, A. P. Davis, *Angew. Chem. Int. Ed.* **2002**, *41*, 4093.

[13] MacroModel 9.9 (MMFFs force field, water GB/SA solvation) accessed via the Maestro 9.2 interface.

[14] For further details, see Supporting Information.

[15] D. H. Leung, R. G. Bergman, K. N. Raymond, *J. Am. Chem. Soc.* **2008**, *130*, 2798.

[16] E. Klein, Y. Ferrand, N. P. Barwell, A. P. Davis, *Angew. Chem. Int. Ed.* **2008**, *47*, 2693.

[17] P. Csermely, R. Palotai, R. Nussinov, *Trends Biochem. Sci.* **2010**, *35*, 539. D. Bucher, B. J. Grant, J. A. McCammon, *Biochemistry* **2011**, *50*, 10530.